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Molecular study of head lice among primary school children in Kirkuk province – Iraq

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Abstract

Head lice are a type of external parasitic insect belonging to the order Phthriaptera. These insects are characterized by their small size and flattened shape. Head lice do not have simple eyes, but the eyes are reduced or completely absent. They also have piercing-sucking mouthparts. These insects live on mammals and feed on their blood, potentially transmitting diseases to humans. Samples were taken from different areas in Kirkuk Governorate. Polymerase chain reaction (PCR) was performed on five DNA samples extracted from head lice parasites to detect the presence of the COX1 gene using primers designed for this purpose. Agarose gel electrophoresis of the PCR products showed the appearance of bands of 720 base pairs in all amplified samples, representing 100% of the studied samples.

1. Introduction:

Lice belonging to the genus Pediculus (Phthiraptera) infect individuals globally. Lice that infest clothing are conventionally referred to as body lice, while those that infest the scalp are termed head lice [1], [2] Head lice predominantly infest youngsters and are considered insignificant as carriers of louse-borne illnesses [3], [4].

Head lice are diminutive, wingless insects that consume human blood and inhabit the scalp. They measure approximately 2 mm to 4 mm in length and possess six legs. Transmission generally occurs through intimate touch between individuals, particularly via their hair [5].

Blood-sucking head lice are classified within the Kingdom Animalia, Class Insecta, Order Phthiraptera, Family Pediculidae, and Genus Pediculus. Species: humanus and subspecies: capitis, an obligate parasite of humans, is highly specialised for bloodsucking and maintains a strong relationship with its host, completing its whole life cycle [6].

There are six possible mitochondrial clades in human lice: A, B, C, D, E, and F. These clades are distributed differently across the world. The other clades are limited to certain regions, in contrast to the broad distribution of Clades A and B. Numerous nations in Asia and Africa have confirmed the presence of Clade C.

It appears that only African lice possess clades D and E, while Amazonian lice have been shown to possess clade F.

With the exception of two groups (B and C), which include entirely of head lice, the remaining groups (A, D, E, and F) comprise both head and body lice [1], [7]. Recent research on pediculosis indicate that Turkey and Iran are disproportionately represented among Asian countries. Head louse infestation rates vary throughout these countries, from just under one percent to 42.6 percent. There is a great deal of variation in pediculosis among Iran's provinces [8], [9], [10].

Molecular identification of head lice is necessary for understanding the spread of diseases transmitted by lice in people and devising effective preventive management methods against these diseases. To better understand the parasite's ability to persist in host populations and for its practical use in evolutionary and basic biology, this information is vital. Given the remarkable genetic plasticity of lice mitochondria, it is hypothesized that molecular markers involving cytochrome c oxidase subunit 1 COX1 genes could shed light on the parasites' evolutionary processes in various populations [1], [11]. The present study examined the COX1 gene of the mitochondrial clades of head lice collected from Iraqi elementary school pupils because there have been few genetic studies and surveys on this subject.

2. Material and Methods:

2.1 Collection of head lice:

The current study was conducted in the city of Kirkuk from November 2023 until the end of May 2024 and focused on head lice infestation (Pediculus humanus capitis) among students in some primary schools in certain districts and subdistricts of Kirkuk Governorate. The ages of the students ranged between 7-13 years.

Head lice cases were diagnosed among students after obtaining official approvals from the Kirkuk Health Department and the Kirkuk Education Directorate. Clinical and visual examinations were conducted for each male and female student in all primary stages from the first primary to the sixth primary stage by examining the scalp, especially the nape, the front of the head and behind the ears [12], using self-illuminating magnifying lenses. The examination lasted for 3-5 minutes for each person to ensure the presence of adult insects, nymphs or eggs (nits). A plastic comb with fine teeth was used, as well as white papers and pieces of white cloth under the head of the examined person to facilitate the distinction of the stages of falling lice.
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2.2 Extraction of genomic DNA:

DNA was isolated and extracted from head lice samples using extraction kits(G-Spin/Korea) stored at room temperature. The preparation for extracting pure DNA from animal tissues, kit number (17045), was used for DNA extraction. The DNA concentration in the sample was measured using the Nano drop spectrophotometer(Nabi/ Korea) technique directly from each extracted sample. The acceptable absorbance at a wavelength of 260/280 for pure DNA concentration ranges between 1.8-2.0 nanometers.

2.3 Determination of concentration and purity for DNA:

The DNA concentration in the sample is estimated using a nanodrop spectrophotometer technique. Then the device has been zeroed, and add (1-2) microliters of each extracted DNA sample directly to the concentration estimation device, then close the cover, press Measure the sample concentration to be (3 -15 ng/ μ) and the purity is estimated through the absorbance (OD) 260/280 nanometers. It establishes the presence of proteins or salts in the sample. The acceptable absorbance at a wavelength of 260/280 for the concentration of pure DNA is between 0.2 and 1.8 nanometers [13].

The type shown in Table 1 was prepared according to the NBCI gene bank. The specific sequence of the gene fragments was determined by IDT Canada in the form of (lyophilized) and at different concentrations in picomoles. The primers were dissolved in distilled water free of ions, where the final concentration was (Pmol / μl (100) and kept at (-20)^o until use. These primers were used to prepare the working solution by taking 10 μl of the stock solution and adding 90 L μ of distilled deionized water (dDH2O) to prepare a concentration of (10 Poml / μl) of the working solution until the reaction occurred.

The optimum conditions (initial dissociation and replication) were determined after conducting several experiments to achieve these conditions. The temperature was changed during the work of the polymerase chain reaction gradient for all samples to choose the optimum conditions. The concentration of the DNA template was changed between (1.5-2) μl microliters, as these two factors are important in the binding of primers.

The optimum conditions for (Initial denaturation) were determined after conducting several experiments for this condition. The temperature was changed by performing (Gradient PCR) for all samples to choose the optimum condition, as well as changing the concentration of the DNA template between (1.5 -2) μ as these two factors are considered important factors in the primer annealing with the complement in the binding of the primers [7]. As shown in Table 2.

Table 1. The primers used in the current study to detect the mitochondrial gene for COX1 .

Primer	Sequence	T_m (^{o}C)	GC (%)	Product size
Forward	5′-GGTCAACAAATCATAAAGATATTG - 3′	57 54.5	720 base pair	
Reverse	5′-TAAACTTCAGGGTGACCAAAAAATCA - 3′	56.6	52.4	

Table 2. The PCR reaction for the COX gene in the primers.

No.	Phase	T_m (^{o}C)	Time	No. of cycle
1-	Initial Denaturation	94^{o}C	5 min.	1 cycle
2-	Denaturation -2	94^{o}C	45sec	
3-	Annealing	57^{o}C	45sec	35 cycle
4-	Extension-1	72^{o}C	45sec	
5-	Extension -2	72^{o}C	7 min.	1 cycle

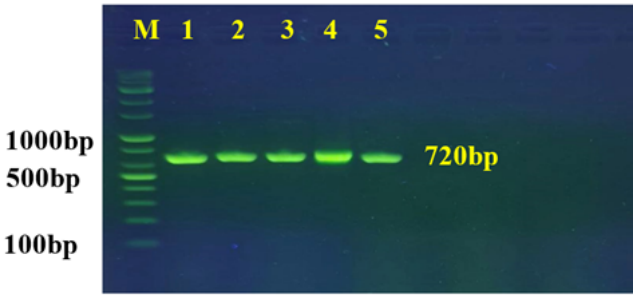

Figure 1. The result of the serial polymerization reaction with a band size of 720 base pairs. The result was obtained by electrophoresis on 1.5% agarose 5 volts/cm.

2.4 Agarose Gel Electrophoresis:

According to the method of [13], a 1.5% agarose gel was prepared and used for electrophoresis in order to determine the PCR product.

3. Results and Discussion:

Genomic DNA was extracted from all isolates of Head lice for use as a starting point for PCR amplification using the supplied extraction kit. The primers are shown in Table 1. Molecular detection of the COX1 gene (720 bp) in the DNA of head lice, prepared according to the NBCI gene bank.

The results of DNA extraction from head lice using specialized equipment for this purpose, electrophoresis with agarose gel, detection using safe Red dye, and examination under ultraviolet light showed the presence of a single molecular size. As shown in Figure 1.

The PCR was performed on 5 samples of DNA extracted from head lice to detect the presence of the COX gene using primers designed for this purpose. Agarose gel electrophoresis of the PCR products revealed bands of 720 base pairs in all amplified samples, representing 100% of the samples studied. A study that was done by [14] showed genomic data on head lice that affected Syrian refugees living in certain camps in Erbil.

According to this study, the parasite has a highly conserved COX gene. This means that its genetic diversity is not affected by local factors or natural selection, which is why it is found all over the world. This method has been used before by [7], [15], [16]. It has been shown in previous studies that variations in gene expression can be attributed to DNA mutations that cause differences in mRNA folding. The present result is comparable to the results plotted from previously isolated sequences by [17], which confirms that there is genetic diversity in the isolated sequence due to slight differences in the number of loops [18].

4. Conclusion:

The present study concluded the presence of the COX-1 gene as 100% percent in head lice among students in some primary schools in certain districts and sub-districts of Kirkuk Governorate.

Funding: None.

Data Availability Statement: All of the data supporting the findings of the presented study are available from corresponding author on request.

Declarations: Conflict of interest: The authors declare that they have no conflict of interest.

Ethical approval: Approval was also obtained from the University of Kirkuk and the Children's Hospital not to take samples from patients suffering from low blood levels, case number 305, dated 5/3 /2024.

Author Contributions: Havin Adel Qadir is responsible for collecting samples, conducting analysis, interpreting data, writing the manuscript, and proofreading it. ∗, Omar Salih Hassan conceived the idea, supervised the research, and read the manuscript.

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دراسة جزيئية لقمل الراس من اطفال المدارس الابتدائية في محافظة كركوك العراق

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الخلاصة

قمل الرأس هو نوع من الحشرات الطفيلية الخارجية التي تنتمي إلى رتبة القمل الماص (*Phthriaptera*). تتميز هذه الحشرات بحجمها الصغير وكونها مفلطحة الشكل. لايمتلك قمل الراس عيونا بسيطة بينما تكون العيون مختزلة او غائبة تماما . كما تتميز باجزاء فم ثاقبة ماصة وتعيش هذه الحشرات على الثدييات وتتغذى على دمائها، وقد تنقل عن طريقها بعض الأمراض للإنسان فقد تم اخذ العينات من مناطق متفرقة في اقضية محافظة كركوك. اجري تفاعل البلمرة المتسلسل على 5 عينات من الحمض النووي المستخلص من طفيلي قمل الرأس للتحري عن وجود الجين *COX*1 باستخدام بوادئ مصممة لهذا الغرض. اظهر الترحيل الكهربائي على هلام الاكاروز لنواتج تفاعل البلمرة المتسلسل ظهور حزم بحجم 720 قاعدة في جميع العينات المضخمة وهو ما يمثل نسبة 100% من العينات المدروسة.

الكلمات الدالة : قمل الراس ؛ جين المايتوكوندريا *COX*1 ؛ الدراسة الجزيئية.

التمويل: لايوجد.

بيان توفر البيانات: جميع البيانات الداعمة لنتائج الدراسة المقدمة يمكن طلبها من المؤلف المسؤول.

اقرارات:

تضارب المصالح: يقر المؤلفون أنه ليس لديهم تضارب في المصالح.

الموافقة الأخلاقية: استُرشد البحث بالمعايير الأخلاقية لإعلان هلسنكي. أُجريت العملية بعد الحصول على موافقة المرضى الشفهية والتحليلية قبل جمع العينات. وقد قامت اللجنة الأخلاقية المحلية في جامعة كركوك بتقييم بروتوكول البحث ومعلومات المشاركين ونموذج الموافقة، واعتمدته.

مساهمات المؤلفين: تولّت أسماء محمد محمود مسؤولية جمع العينات، وتحليل البيانات، وتفسيرها، وتأليف المخطوطة، وتدقيقها. أما سعدية شهاب حمد وهنادي عبد الإله عبد الرزاق، فقد ابتكرتا الفكرة، وأشرفتا على البحث، وراجعتا المخطوطة.